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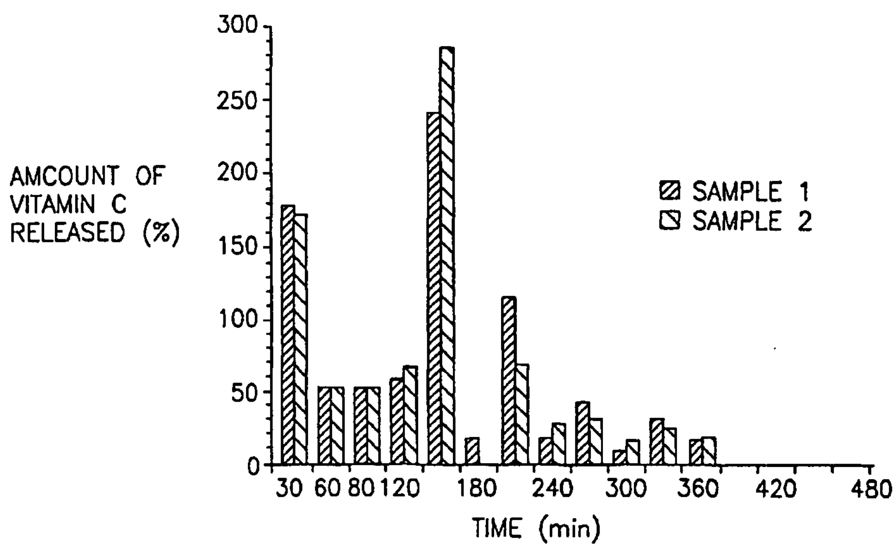


(57) Abstract

The present invention provides a composition for oral delivery of a bioactive agent to a subject. The composition comprises three delivery forms of the bioactive agent, at least two of which being in the form of microcapsules having each an external coating encapsulating the agent. The bioactive agent is released in the digestive tract in essentially three release pulses since the coating of the microcapsules of each form is different than that of the other form or forms.

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# PULSE-DELIVERY ORAL COMPOSITIONS

## FIELD OF THE INVENTION

This invention relates in general to compositions for the delivery of bioactive agents to a subject. In particular it describes a composition which delivers a bioactive agent in a pulsative manner and is intended for oral administration.

## BACKGROUND OF THE INVENTION

The use of microencapsulation to protect sensitive bioactive agents from degradation has become well known. Typically, a bioactive agent is encapsulated within any of a number of protective wall materials, usually polymeric in nature. The agent to be encapsulated can be coated with a single wall of polymeric material or can be homogeneously dispersed within a polymeric matrix. The amount of agent inside the microcapsule can be varied as desired, ranging from either a small amount to as high as 95% or more of the microcapsule composition. The diameter of the microcapsule can also be varied as desired, ranging from less than one micrometer to as large as three millimeters or more.

EP 199362 and U.S. patents Nos. 4,900,556 and 4,921,757 describe a system for delayed and pulsed release of biologically active substances. The active substance is entrapped in encapsulated liposomes which are stimulated by external factors or by including a phospholipase within the liposomes, at distinct intervals of time to produce a pulsed release of the entrapped substance.

Vitamin C (ascorbic acid) is an example of a bioactive agent, which its presence in the body is needed at substantial high concentrations but at the same time is not entirely absorbed when administered in a single dose.

Vitamin C is involved in a great number of biochemical reactions in the

human body. Two of its major interactions are in potentiating the immune system and aiding the synthesis of the protein collagen, which is a very important substance that strengthens the blood vessels, the skin, the muscles and the bones.

The clinical effects of metabolic reactions in which vitamin C is involved have been widely recognized and reported. For example, the free-radical scavenging effect is believed to enable the body to convert carcinogens to non-toxic derivatives which are eliminated in the urine and, consequently, to ameliorate the effects of smoking and exposure of the body to other environmental pollutants.

The establishment and maintenance of effective levels of vitamin C and its derivatives in the human body yield important health advantages. While most animals have a liver enzyme which enables them to manufacture vitamin C in situ by conversion of blood sugar into ascorbic acid, humans do not have this enzyme. As a consequence, the vitamin C which is required by the human body for the various metabolic reactions must be ingested with the human diet. Furthermore, the human body does not have the ability to store vitamin C and it is excreted if unmetabolized. Low constant levels of vitamin C and its derivations in the human body induce a variety of undesirable physiological responses.

Given the large number of "free radical" diseases, it is reasonable to assume that taking an optimal dose of vitamin C could be extremely beneficial, as suggested by Harman D. et al., in "Free Radical Theory of Aging: Current Status", Lipofuscin 1987; State of the Art, edited by I. Zs. Nagy, New York, Elsevier, 1988, p. 3-21. Because large doses are not entirely absorbed and may even irritate a patient's stomach, and because small doses may be insufficient to provide adequate anti-oxidant protection, there exists a need to a system for delivering vitamin C in a dosage sufficiently large to ensure as large as possible a presence in the body while at the same time minimizing excesses and waste through excretion.

Vitamin C absorption by man has been studied, see for example Thielemann A. M. et al, in Il Farmaco – Ed. Pr., vol. 43, 1988. According to this article, an increase in the absorption has been observed on administration of vitamin C in separate doses, compared with a single dose. Therefore, it was expected that a

similar effect could be achieved by administering the vitamin in a slow release preparation. However, no statistical difference was observed with the urinary excretion of vitamin C between a slow release preparation and an aqueous solution of the same 1 gr. dose (Anderson T.W. et al., CMA Journal, 5,823,1975).

## SUMMARY OF THE INVENTION

It is an object of the present invention to provide an oral composition that releases certain amounts of a bioactive agent from one single administered dose, in three pulses. It is proposed that such a release would prevent carrier saturation and provide a better contact of the bioactive agent with the absorption sites, thus improving its efficiency and preventing excess loss via degradation and/or excretion.

The above object is achieved according to the present invention, by a composition for the oral delivery of a bioactive agent to a subject, comprising three delivery forms of the bioactive agent, at least two of which being in the form of microcapsules having each an external coating encapsulating said agent, characterized in that the coating of the microcapsules of each form is different than that of the other form or forms, said agent being released in the digestive tract in essentially three release pulses.

The bioactive agent used in the compositions of the present invention is either one that its presence in the body is needed at substantial high concentrations but at the same time is not entirely absorbed when administered in a single dose or it is an agent that should be administered in a pulsative manner in order to ensure therapeutical activity . A preferred bioactive agent is vitamin C.

According to a preferred aspect, the three release pulses of the bioactive agent are provided by the composition of the present invention, wherein the three delivery forms are microcapsules. The coating of a first group of microcapsules is gastrosoluble, the coating of a second group of microcapsules is enterosoluble and the coating of the third group of microcapsules is enterosoluble but with dissolution properties in an intestinal or intestinal-like medium such that it releases the

bioactive agent in the intestine at slower rate than the second group of microcapsules.

According to another preferred aspect, the three release pulses of the bioactive agent are provided by the composition of the present invention, wherein one delivery form of the bioactive ingredient is other than a microcapsule and is gastrosoluble, the remaining two delivery forms being microcapsules which have distinct dissolution properties in an intestinal or intestinal-like medium.

## DETAILED DESCRIPTION OF THE INVENTION

The composition of the present invention comprises of different forms of a bioactive agent, designed so as to release the bioactive agent in the stomach and in the intestines at different time intervals. Examples of bioactive agents are vitamins such as vitamin C, or drugs such as amoxycillin, ampicillin, tetracycline, nalidixic acid, cefaclor, cephalexin, clindamycin, erytromycin, verapamil, omeprazole, ciprofloxacin, famotidine, diclofenac sodium, nifedipine, ceftriaxone sodium, dilthiazem hydrochloride, captopril, lansoprazole, cefuroxime axetil, albuterol sulfate, metoprolol succinate or pentoxifylline. A preferred bioactive agent is vitamin C and the invention will be further described with reference to vitamin C.

The gradual release of the active substance is achieved by using particles of the bioactive agent that have different solubilities in the gastrointestinal tract, i.e. by using in the same system gastrosoluble particles that are soluble under acidic conditions, together with enterosoluble particles.

More preferably, in order to obtain a system with three release pulses, the enterosoluble particles themselves should vary in their solubility in the intestines, so as to have one group of such particles that dissolves quickly in the intestinal fluid and a second group that dissolves at a slower rate. Therefore, according to a preferred embodiment, the system of the present invention contains a blend of three groups of particles: a group that releases vitamin C in the stomach, a second group

that releases vitamin C quickly in the intestines and a third group that releases vitamin C slowly in the intestines.

To achieve pulse delivery of a bioactive agent, microencapsulation technology is used. This technology leads to the formation of individualized particles coated with an appropriate polymer. The advantages gained by the encapsulation of a bioactive agent with a polymer are multi-fold and include: protection against humidity, chemical agents or extreme thermal conditions; taste and color masking; modification of the surface, improved fluidity and flowing properties of powders; controlled or prolonged release of drugs using predefined release mechanisms such as:

• diffusion through a non-degraded polymer envelop

• release through (bio)degradation of the polymer coating (chemical, physical, mechanical, enzymatic or by a combination of these effects)

• controlled dissolution of the coating polymer in the release medium.

The gastrosoluble particles used in the composition of the present invention may be either encapsulated or not encapsulated in case that the bioactive material has a good solubility in the stomach. Coating materials for the production of gastrosoluble microcapsules may be selected from commercially available acid-soluble coating materials such as acrylic resins (e.g. Eudragit ®E100) or methacrylic resins.

The enterosoluble coating materials that dissolve quickly in an intestinal or intestinal-like medium may be selected from: cellulose derivatives such as hydroxypropylmethylcellulose phthalate (e.g. HPMCP 55), hydroxypropyl methylcellulose acetate succinate, hydroxypropyl methylcellulose (e.g. Pharmacoat® 606), carboxymethyl ethyl cellulose (e.g. Duodcell® AQ), cellulose acetate phthalate, or other polymers such as polyvinyl acetate phthalate.

Examples of enterosoluble coating materials that have dissolution properties in an intestinal or intestinal-like medium such that release the bioactive agent in the intestine at a slower rate are copolymers of methacrylic acid and

methyl methacrylate and/or methyl acrylate that are soluble under pH values higher than 7 (e.g. Eudragit® S, Eudragit® 4110D).

The release of vitamin C from microcapsules is dictated by the solubility of the coating materials in the gastrointestinal tract. However, certain additives may affect the solubility and the coating properties of a certain coating material, such as for example surfactants, plasticizing agents and brittleness-inducing agents. The plasticizing agents can be selected from commercially available agents, and preferred plasticizing agents are diethyl phthalate, dibutyl phthalate, triethyl citrate, polyethylene glycol, acetyl triethyl cytrate, dibutyl sebacate, or glyceryl triacetate. The effective amount of the plasticizing agent varies between 2 and 20% of the total dry substance of the coating material.

A brittleness-inducing agent is defined as a dose agent which decreases the elasticity of the film which forms the coatings. Examples of commercially available brittleness-inducing agents are talc, aerosil and magnesium stearate and effective amounts are 5 to 60% relative to the total dry substance of the coating material.

The encapsulation of a bioactive agent may be carried out by various known methods, for example spray-drying, spray-coating, solvent emulsion evaporation or extraction, prilling, extrusion or supercritical $CO_2$. The spray-coating technique is especially preferred, since it is easily scaled up and is well adapted to obtain microparticles whose diameter ranges between 100 and 500 μm.

A typical process for the preparation of encapsulated bioactive agents, for example of vitamin C, includes the steps of placing vitamin C particles in a fluidized hot air and carrying out spray-coating onto the vitamin C particles, using a polymer solution/dispersion so as to produce a microcapsule wall at the surface of vitamin C. The air temperature depends on the polymer solvent evaporation temperature. The polymer solution/dispersion is kept under continuous stirring and pumped up to the spray nozzle at the bottom of the

fluidized air bed. The polymer solution may be sprayed in a number of separate periods, and between each spraying the particles are cured. After the last curing step, the vitamin C microcapsules are harvested.

The compositions of the present invention may normally be for pharmaceutical and/or nutritional use and in a solid form, e.g. with the microparticles/microcapsules incorporated in a unit dose such as tablets, capsules, including hard gelatine capsules, caplets, chewable tablets, tongue tablets, as a mixture in a sachet, or in any adequate nutritive form, for example bars, cereals, etc.

The composition of the present invention may further comprise one or more food additives or ingredients which strengthen or boost the immune system or other body functions, as for example propolis, echinacea, minerals, amino acids, ginseng, antioxidants, etc.

The invention will now be described in greater detail in the following non-limiting Examples with reference to the accompanying drawings, in which:

**Fig. 1** - a graph showing the stability profile of 2g/L vitamin C solutions versus time at 37$^0$C, in an acidic or neutral medium

**Fig. 2** – a graph showing the in vitro release profile of vitamin C recorded for batch A at 37$^0$C, in an acidic medium (pH=1.2)

**Fig. 3** - a graph showing the in vitro release profile of vitamin C recorded for batch B for 2 hours in an acidic medium and then 6 hours in a neutral medium

**Fig. 4** – a graph showing the release profile of batch D, HPMCP 55 + 25% talc for 2 hours in an acidic medium, 6 hours in a neutral medium

**Fig. 5** – a graph showing the in vitro release profile of batch G (Eudragit® RS30D) for 2 hours in an acidic medium and 5 hours in a neutral medium

**Fig. 6** – a graph showing the in vitro release profile of batch H (Eudragit ® 4110D + 10% talc), for 2 hours in an acidic medium, and 6 hours in a neutral medium

**Fig. 7** – a graph showing the in vitro release profile of batch I, (Eudragit ® 4110D + 25% talc) for 2 hours in an acidic medium, 6 hours in a neutral medium

**Fig. 8** – a graph showing the in vitro release profile of Batch 1 (uncoated vitamin C), Batch 2 (HPMCP + 25% talc) and Batch 3 (Eudragit ® 4110D + 25% talc)

**Fig. 9** – a graph showing the release profile of vitamin C recorded for PDS 1 for two hours in an acidic medium and 6 hours in a neutral medium

**Fig. 10** - a graph showing the release profile of vitamin C recorded for PDS 2 for two hours in an acidic medium and 6 hours in a neutral medium

**Fig. 11** - a graph showing the release profile of vitamin C recorded for PDS 3 for two hours in an acidic medium and 6 hours in a neutral medium

**Fig. 12** - a histogram showing the amount of vitamin C released versus time for the PDS 3 containing 800 mg. of vitamin C

**Fig. 13** - a graph showing the release profile of vitamin C recorded for PDS 4 for two hours in an acidic medium and 6 hours in a neutral medium

**Fig. 14** - a histogram showing the amount of vitamin C released versus time for the PDS 4 containing 800 mg. of vitamin C

**Fig. 15** - a graph showing the release profile of vitamin C recorded for PDS 5 for two hours in an acidic medium and 6 hours in a neutral medium

**Fig. 16** - a histogram showing the amount of vitamin C released versus time for the PDS 5 containing 800 mg. of vitamin C

**Fig. 17** - a graph showing the release profile of vitamin C recorded for PDS 6 for two hours in an acidic medium and 6 hours in a neutral medium

**Fig. 18** - a histogram showing the amount of vitamin C released versus time for the PDS 6 containing 800 mg. of vitamin C

**Fig. 19** – a graph showing the in vitro release profile of a Twinlab® tablet (C-1000 Tabs)

## EXPERIMENTAL

### I. General

Vitamin C particles are commercially available from Hoffmann-La-Roche. CME sieved all the particle batches, to only keep particles whose diameter is higher than 200 μm. These particles are well adapted to the spray-coating method.

The measurement of vitamin C content in the microcapsules was performed using a UV spectrophotometer. A known amount of microcapsules was ground so as to break the capsule wall and release the vitamin C. The ground microcapsules were introduced in the appropriate dissolution medium chosen to mimic either a gastric or an enteric medium. The amount of vitamin C was measured using a UV spectrophotometer. Stability of vitamin C in the dissolution medium was preliminary checked.

### II. In Vitro Release Profile

The measurement of vitamin C released from the particles at given time intervals was performed using a UV spectrophotometer. A known amount of microcapsules was introduced in a dissolution medium, either an acidic solution at pH=1 as the gastric fluid, or a neutral solution at pH=6.8 as the intestinal fluid.

The UV absorbance of the dissolution medium in which the microcapsules were incubated, was measured and compared with that of reference solution samples (calibration curve) to determine the vitamin C concentration in the solution, and then the amount of vitamin C released in vitro from the microcapsules versus time.

## II. 1 Release Profile of Vitamin C

For the development of the pulse-delivery system, the release profile of vitamin C from microcapsules was recorded successively for 2 hours in the acidic medium and 6 hours in the neutral solution.

The vitamin C-containing microcapsules were placed in the acidic medium under stirring, nitrogen and protection from light for 2 hours. Then, the microparticles were separated by filtration and placed in the citrate buffer solution (pH=6.8) under stirring, nitrogen and protection from light, to determine the Vitamin C profile in the neutral medium.

Figure 1 shows the stability profile of two citrate buffer solution and two acidic media containing 2g/L Vitamin C versus time at $37^0$C. The measurement has been performed successively for two different samples, so as to check the reproductibility of each assay.

## II.2 Vitamin C coated with a gastrosoluble polymer

Coating Material: Eudragit ®E 100

Experimental Conditions:

The batch A produced in this experiment is based on Vitamin C coated with a gastrosoluble polymer: Eudragit ®E. The in vitro release profile of this batch is shown in Fig. 2.

Figure 2 shows the in vitro release profile of Vitamin C recorded in an acidic medium. The measurement has been performed successively for two different samples, so as to check the reproducibility of the assay. From Fig.2 it can be noticed that all the Vitamin C contained in the microcapsules is solubilized rapidly (15 min. ) in an acidic medium. The Eudragit ® E100 coating allows a rapid vitamin C dissolution in an acidic medium.

## II.3 Vitamin C coated with an entersoluble polymer

### II.3.1 Coating material: HPMCP 55.

The objective of the trial with HPMCP 55 is to find a coating that is stable in the stomach.

Experimental Conditions:

The coating solution composition has been fixed as follows:

**Table 1**

| | Product | Amount (g) | Weight % |
|---|---|---|---|
| Polymer | HPMCP 55 | 198 | 8.26 |
| Plasticizer | Diethyl Phthalate | 20 | 0.83 |
| Solvents | Isopropanol | 545 | 22.73 |
| | Ethyl Acetate | 1635 | 68.18 |

The selected process parameters for the trials are presented in Table 2:

**Table 2**

| Parameters | Conditions |
|---|---|
| Inlet Air Temperature | $60^0C$ |
| Outlet air temperature | $49^0C$ |
| Spraying pressure | 1 bar |
| Polymer solution flow rate | 4.2 mL/min |
| Weight of vitamin C (d>200μm) | 500 g |

The polymer solution has been sprayed in 4 separate periods. Between each spraying step, the particles have been cured for half an hour, so as to form a continuous polymer film on the Vitamin C particles. The reference of this batch is batch B.

**In vitro release profile**

Figure 3 shows the in vitro release profile of vitamin C recorded for batch B for 2 hours in an acidic medium and then 6 hours in a neutral medium. The measurement has been performed successively for two different samples, so as to check the reproductibility of the assay.

Fig. 3 shows that 55% of the vitamin C contained in the mircrocapsules is solubilized after two hours in an acidic medium. The remaining vitamin C contained in the microcapsules is rapidly solubilized in the neutral medium. Furthermore, the batch seems to be relatively homogeneous. The release profile is quite similar for two different samples taken from the same batch.

II.3.2 Coating material: HPMCP55 and talc

In order to improve the efficiency of the coating film, talc was added.

***HPMCP55 and 25% talc**

The coating solution composition has been fixed as follows:

**Table 3**

| | Product | Amount (g) | Weight % |
|---|---|---|---|
| Polymer | HPMCP55 | 161.21 | 7.51 |
| | Talc | 39.73 | 1.85 |
| Plasticizer | Diethyl Phthalate | 15.69 | 0.73 |
| Solvents | Isopropanol | 482.98 | 22.51 |
| | Ethyl acetate | 1446.32 | 67.40 |

The polymer solution (5mL/min flow rate) has been sprayed in four separate periods, at a spraying pressure of 1 bar. Between each spraying step, the particles have been cured for half an hour, so as to form a continuous polymer film on the vitamin C particles (weight of vitamin C – 500 g).

***HPMCP55 and 50% talc**

The coating solution composition is presented in Table 5:

**Table 5**

| | Product | Amount (g) | Weight % |
|---|---|---|---|
| Polymer | HPMCP55 | 136.25 | 6.25 |
| | Talc | 68.13 | 3.13 |
| Plasticizer | Diethyl Phthalate | 13.62 | 0.62 |
| Solvents | Isopropanol | 490.5 | 22.5 |
| | Ethyl acetate | 1471.5 | 67.5 |

The polymer solution (5.5 mL/min flow rate) has been sprayed in five separate periods, at a spraying pressure of 1 bar. Between each spraying step, the particles have been cured for half and hour, so as to form a continuous polymer film on the Vitamin C particles (weight of vitamin C – 500 g).

Figure 4 shows the release profile of vitamin C recorded for batch D (HPMCP55 and 25% talc) for 2 hours in an acidic medium and 6 hours in a neutral medium. The measurement has been performed successively for two different samples, so as to check the reproductibility of the assay.

It appears that the increase of talc content leads to a little bit slower release of vitamin C in the acidic medium. For this reason, a 50% talc content - batch E, in the coating has been evaluated. For batch E, it was noticed that 60% of the vitamin C contained in the microcapsules is solubilized after two hours in an acidic medium and the remaining vitamin C is solubilized rapidly in the neutral medium.

From these experiments it was concluded that the talc content in the coating film has to be adjusted at 25% to get the slowest dissolution rate.

**Conclusion**

The best results as regards the release profile of Vitamin C in the intestine were achieved with HPMCP55 and 25% talc. Under these conditions, only 32%

of the vitamin C contained in the microcapsules is solubilized in the acidic medium and the remaining vitamin C is rapidly solubilized in the neutral medium.

II.3.3 Vitamin C coated with a wax and an enterosoluble polymer

The Eudragit® 4110D dispersion formulation prepared is shown in Table 5:

**Table 5**

| | Product | Amount (g) | Weight % |
|---|---|---|---|
| Polymer | Eudragit | 714.26 | 20 |
| | Talc | 4.28 | 0.4 |
| Plasticizer | Triethylcitrate | 6.43 | 0.6 |
| Solvent | Water | 346.43 | 79 |

This dispersion was sprayed onto the Vitamin C particles already coated with stearic acid. The process parameters selected for the trials are presented in Table 6.

**Table 6**

| Parameters | Conditions |
|---|---|
| Inlet Air Temperature | $45^0C$ |
| Outlet air temperature | $36^0C$ |
| Spraying pressure | 1 bar |
| Polymer solution flow rate | 4.5 mL/min |
| Weight of vitamin C (d>200μm) | 500 g |

The polymer solution has been sprayed in 6 steps. Between each spraying step, the particles have been cured for half an hour so as to favor the formation of a continuous polymer film on the vitamin C particles.

**In vitro release profile**

It appears that the stearic acid + Eudragit ® 4110D coating prepared is not able to prevent Vitamin C dissolution in an acidic medium. Stearic acid does not improve the efficiency of the coating material Eudragit ® 4110D. However, it is worthwhile noticing that the release profile of the Vitamin C in the neutral medium is slower with Eudragit ® 4110D than with HPMCP55.

**II.4 - Sustained release of Vitamin C in a neutral medium**

The aim of these trials is to produce coated vitamin C particles, which release vitamin C in a neutral medium very slowly, in a sustained way.

The first polymer tested is the pH-independent coating polymer Eudragit® RS30D. The second polymer is Eudragit ® 4110D blended with talc to increase the release time of Vitamin C.

**Coating material: Eudragit ® RS30D**

Eudragit ® RS30D is an aqueous dispersion of a copolymer of an amino alkyl methacrylate monomer and acrylic and methacrylic esters.

Experimental conditions

The coating dispersion composition has been fixed as follows:

**Table 7**

| | Product | Amount (g) | Weight % |
|---|---|---|---|
| Polymer | Eudragit ® RS30D | 420.17 | 39.22 |
| | Talc | 25.21 | 2.35 |
| Plasticizer | Triethylcitrate | 63.02 | 5.88 |
| Solvent | Water | 563.01 | 52.55 |

The polymer dispersion (10 mL/min flow rate) was sprayed in four separate periods at a spraying pressure of 1 bar. Between each spraying step, the particles have been cured for half an hour, so as to form a continuous polymer film on the Vitamin C particles (weight of vitamin C – 500 g).

**In vitro release profile**

Figure 5 shows the in vitro release profile of vitamin C recorded for this batch (batch G) for 2 hours in an acidic medium and 5 hours in a neutral medium. The measurement has been performed successively for two different samples, so as to check the reproductibility of the assay.

**Coating materials: Eudragit ® 4110D and 10% talc**

Experimental conditions

- **Eudragit ® 4110D and 10% talc (batch H)**

The coating dispersion composition has been fixed as follows:

**Table 8**

| | Product | Amount (g) | Weight % |
|---|---|---|---|
| Polymer | Eudragit ® 4110D | 632.1 | 59.0 |
| | Talc | 18.96 | 1.77 |
| Plasticizer | Triethylcitrate | 5.69 | 0.53 |
| Solvent | Water | 414.65 | 38.7 |

The selected process parameters for the trials are presented in **Table 9**:

| Parameters | Conditions |
|---|---|
| Inlet Air Temperature | 45$^0$C |
| Outlet air temperature | 37$^0$C |
| Spraying pressure | 1 bar |

| | |
|---|---|
| Polymer solution flow rate | 4-5 mL/min |
| Weight of vitamin C (d>200μm) | 500 g |

The polymer dispersion was sprayed in four separate periods. Between each spraying step, the particles have been cured for half an hour so as to form a continuous polymer film on the Vitamin C particles.

**In vitro release profile**

Figure 6 shows the release profile of vitamin C recorded for batch H for 2 hours in an acidic medium and 6 hours in a neutral medium. The measurement has been performed successively for two different samples, so as to check the reproductibility of the assay.

It can be observed that 25% of the vitamin C contained in the microcapsules is solubilized after two hours in an acidic medium. The remaining Vitamin C contained in the microcapsules is totally solubilized after three hours in the neutral medium. Hence, it appears that the dissolution of Vitamin C particles in neutral medium has been extended over a longer period of time.

- **Eudragit ® 4110D and 25% talc (batch I)**

The coating dispersion composition has been fixed as follows:

**Table 10**

| | Product | Amount (g) | Weight % |
|---|---|---|---|
| Polymer | Eudragit ® 4110D | 558.03 | 26.05 |
| | Talc | 41.85 | 1.95 |
| Plasticizer | Triethylcitrate | 5.02 | 0.23 |
| Solvent | Water | 1537.9 | 71.77 |

The selected process parameters for the trials are presented in **Table 11**:

| Parameters | Conditions |
|---|---|
| Inlet Air Temperature | $45^0$C |
| Outlet air temperature | $34^0$C |
| Spraying pressure | 1 bar |
| Polymer solution flow rate | 5 mL/min |
| Weight of vitamin C (d>200μm) | 500 g |

The polymer dispersion was sprayed in four separate periods. Between each spraying step, the particles have been cured for half an hour so as to form a continuous polymer film on the vitamin C particles.

**In vitro release profile**

Figure 7 shows the release profile of Vitamin C recorded for batch I for 2 hours in an acidic medium and 6 hours in a neutral medium. The measurement has been performed successively for two different samples, so as to check the reproductibility of the assay.

From Fig. 7 it can be observed that only 10% of the Vitamin C contained in the microcapsules is solubilized after two hours in an acidic medium. The remaining Vitamin C contained in the microcapsules is totally solubilized after two hours in the neutral medium. Hence, it appears that the dissolution rate of Vitamin C particles in the acidic medium has been strongly decreased. In the neutral medium, Vitamin C is then totally solubilized after two hours.

## III. Development of a Pulse Delivery System

Vitamin C without any coating was used for the first batch, if no specific coating is required to enhance stability of the product during the storage period.

As regards the second batch, the appropriate coating material was an entersoluble polymer: HPMCP55, blended with 25% talc. This composition, at a 35% coating ratio, makes it possible to release 32% of vitamin C in two hours in an acidic medium and the remaining Vitamin C as a single dose pulsed in the neutral medium. The talc ratio could even be adjusted to restrict the dissolution of vitamin C in the acidic medium.

The third batch was the slow and sustained release form of vitamin. The coating composition elaborated is made of the polymer Eudragit® 4110D with 25% talc. Under these condition, 10% of vitamin C is released after two hours in an acidic medium and the remaining vitamin C after three hours in the neutral medium.

The talc ratio could even be adjusted to extend the dissolution time of vitamin C contained in the microcapsules.

To produce a pulse-delivery system, the composition of the three batches to be prepared is as follows:

-Batch 1: Vitamin C without coating

-Batch 2: Vitamin C coated with HPMCP55 and 25% talc

- coating ratio: 35%
- amount of vitamin C solubilized after two hours in the acidic medium:32%
- pulse of the remaining vitamin C in the neutral medium (15 minutes)

-Batch 3: Vitamin C coated with Eudragit ® 4110D and 25% talc

- coating ratio: 36%
- amount of vitamin C solubilized after two hours in acidic medium: (10%)
- remaining vitamin C solubilized in neutral medium over a period of 3 hours

Figure 8 shows the in vitro release profile of the three batches proposed.

Six blends of the selected batches were prepared with different contents of particles from each batch, so as to record and compare the release profile of the potential pulse-delivery systems. The compositions are presented in Table 12.

**Table 12**

| Pulse-delivery system (PDS) | Batch 1 (part) | Batch 2 (part) | Batch 3 (part) |
|---|---|---|---|
| 1 | 1 | 1 | 1 |
| 2 | 1 | 2 | 3 |
| 3 | 1 | 3 | 2 |
| 4 | 0.5 | 4.5 | 1 |
| 5 | 0.5 | 4 | 1,5 |
| 6 | 0.5 | 3.5 | 2 |

- **First System (1,1,1)**

Figure 9 shows the release profile of Vitamin C recorded for this first PDS for two hours in an acidic medium and 6 hours in a neutral medium. The measurement was performed successively for two different samples, so as to check the reproductibility of the assay.

**In vitro release profile of PDS 1**

**(Vitamin C uncoated/HPMCP55 + 25% talc / Eudragit ® 4110D+25% talc: 1/1/1)**

From Fig. 9 it can be observed that more than 60% of the Vitamin C contained in this system is solubilized after two hours in the acidic medium. The remaining Vitamin C contained in the system is solubilized after one hour in the neutral medium. The second pulse does not appear in the release profile. For this

reason, it has been concluded that the amount of Batch 2 in the system has to be increased.

- **Second system (1,2,3,)**

Figure 10 shows the release profile of vitamin C recorded for the second PDS for two hours in an acidic medium and 6 hours in a neutral medium. The measurement has been performed successively for two different samples, so as to check the reproductibility of the assay.

**In vitro release profile of PDS 2**

**(Vitamin C uncoated/HPMCP55 + 25% talc/Eudragit ® 4110D + 25% talc: 1/2/3)**

From Fig. 10 it can be observed that 50% of the vitamin C contained in this system is solubilized after two hours in an acidic medium. The remaining Vitamin C contained in the system is solubilized after one hour and a half in the neutral medium. Once again, the second pulse does not appear in the release profile. The content of batch 2 in the blend must be more increased.

- **Third System (1,3,2)**

Figure 11 shows the release profile of vitamin C recorded for the third PDS for two hours in an acidic medium and 6 hours in a neutral medium. The measurement was performed successively for two different samples, so as to check the reproductibility of the assay.

**In vitro release profile of PDS 3**

**(Vitamin C uncoated/HPMCP55 + 25% talc Eudragit® 4110D + 25% talc: 1/3/2)**

From Fig. 11 it can be observed that less than 50% of the vitamin C contained in this system is solubilized after two hours in an acidic medium. The remaining vitamin C contained in the system is solubilized after two hours in the neutral medium. Three pulse-delivery effects clearly appear in the release profile.

Now, the ratio of the different batches in the blend has to be adjusted to obtain three equivalent pulses.

Figure 12 shows a histogram which displays the amount of vitamin C released versus time for the PDS 3 containing 800 mg. of vitamin C.

This figure confirms the achievement of a pulse-delivery system with three steps: vitamin C released immediately in the stomach (30 min.), vitamin C released immediately in the intestines (150 min) and vitamin C released in the intestines slowly (210-240 min).

- **Fourth system (0.5, 4.5, 1)**

Figure 13 shows the release profile of vitamin C recorded for the fourth PDS for two hours in an acidic medium and six hours in a neutral medium. The measurement was performed successively for two different samples, so as to check the reproductibilty of the assay.

**In vitro release profile of PDS 4**

**(Vitamin C uncoated/HPMCP55 + 25% talc/Eudragit ® 4110D + 25% talc: 0.5/4.5/1**

From Fig. 13 it can be observed that 55% of the vitamin C contained in this system is solubilized after two hours in an acidic medium. The remaining vitamin C contained in the system is solubilized after three hours in the neutral medium. Three pulses clearly appear in the release profile. However, the third one is too smooth. For this reason, the amount of batch 3 in the system has to be increased.

Figure 14 shows a histogram which displays the amount of vitamin C released versus time for PDS 4 containing 800 mg. of vitamin C. This figure confirms the achievement of a pulse-delivery system with three steps: vitamin C released immediately in the stomach (30 min.), vitamin C released immediately in the intestines (150 min) and vitamin C released in the intestines slowly (300 min.).

The ratio of the different batches in the system should be adjusted to obtain three equivalent pulses, and more particularly to increase the third one.

- **Fifth System (0.5,4,1.5)**

Figure 15 shows the release profile of vitamin C recorded for the fifth PDS for two hours in an acidic medium and six hours in a neutral medium. The measurement was performed successively for two different samples, so as to check the reproducibility of the assay.

From Fig. 15 it can be observed that 60% of the vitamin C contained in this system is solubilized after two hours in an acidic medium. The remaining vitamin C contained in the system is solubilized after three hours in the neutral medium. Three pulses clearly appear in the release profile. However, the amount of vitamin C released in an acidic medium is still too large.

Figure 16 shows a histogram which displays the amount of vitamin C released versus time for PDS 5 containing 800 mg. of vitamin C. This figure confirms the achievement of a pulse-delivery system with three steps: vitamin C released immediately in the stomach (30 min), vitamin C released immediately in the intestines (150 min) and vitamin C released in the intestines slowly (210-240 min.).

The ratio of the different batches in the system can once again be adjusted to obtain three equivalent pulses, and more particularly to decrease the first one.

- **Sixth system (0.5,3.5,2)**

Figure  shows the release profile of vitamin C recorded for the sixth PDS for two hours in an acidic medium and six hours in a neutral medium. The measurement was performed successively for two different samples, so as to check the reproducibility of the assay.

From Fig. 17 it can be observed that 45% of the vitamin C contained in this system is solubilized after two hours in an acidic medium. The remaining vitamin C contained in the system is solubilized after three and a half hours in

the neutral medium. Three pulses is the release profile, and the time for complete release is ~ 6 hours.

Figure 18 shows a histogram which displays the amount of vitamin C released versus time for PDS 6 containing 800 mg of vitamin C. This figure confirms the achievement of a pulse-delivery system with three steps: vitamin C released immediately in the stomach (30 min.), vitamin C released immediately in the intestines (150 min) and vitamin C released in the intestines slowly (210 min.).

This PDS is the most efficient one, three pulses clearly appear in the release profile and all the vitamin C contained in this system is solubilized within about 6 hours.

**Comparison Test**

For comparison, the release profile of a Twinlab ® tablet (C-1000 TABS, manufactured by TWIN LABORATORIES INC., Ronkonkoma, New York), was evaluated for two hours in an acidic medium and six hours in a neutral medium. A Twinlab ® tablet (weight: 1300 mg.) contains 1000 mg. vitamin C.

Figure 19 shows the release profile recorded. It can be observed that the vitamin C contained in the tablet is solubilized almost linearly versus time over a period of more than 8 hours. This tablet gives a sustained release of vitamin C, but not a pulse-delivery effect.

**CLAIMS:**

**1.** A composition for oral delivery of a bioactive agent to a subject, comprising three delivery forms of the bioactive agent, at least two of which being in the form of microcapsules having each an external coating encapsulating said agent, characterized in that the coating of the microcapsules of each form is different than that of the other form or forms, said agent being released in the digestive tract in essentially three release pulses.

**2.** A composition according to Claim 1, characterized in having a release profile of said agent, when tested *in vitro* under initial acidic conditions at a pH of between about 1 and subsequent neutral conditions, with three release pulses of said active ingredient over a period of from about 2 hours to about 8 hours.

**3.** A composition according to claim 1 wherein the bioactive agent is vitamin C.

**4.** A composition according to any one of claims 1 to 3 wherein the coating of the microcapsule of one or two of the at least two delivery forms is gastrosoluble and that of one or two of the other at least two delivery forms is enterosoluble.

**5.** A composition according to any one of claims 1 to 3 wherein said three delivery forms are microcapsules.

**6.** A composition according to claim 5, characterized in that the coating of a first group of microcapsules is gastrosoluble, the coating of a second group of microcapsules is enterosoluble and the coating of the third group of microcapsules is enterosoluble but with dissolution properties in an intestinal or intestinal-like medium such that it releases the bioactive agent in the intestine at slower rate than said second group.

**7.** A composition according to claim 1 wherein one delivery form of the active ingredient is other than a microcapsule.

**8.** A composition according to any one of claims 1 to 7, wherein the bioactive agent is needed in the subject's body at an amount that is not entirely absorbed when administered in a single dose.

**9.** A composition according to any of the preceding claims, further comprising one or more food additives or ingredients which strengthen or boost the immune system or other body functions.

**10.** A composition according to any one of claims 4 to 6, wherein the gastrosoluble coating is formed of acid-soluble polymers optionally in combination with pharmaceutically acceptable carriers.

**11.** A composition according to claim 10, wherein the acid-soluble polymers are acrylic resins or methacrylic resins.

**12.** A composition according to any one of claims 4 to 6, wherein the coating of the enterosoluble microcapsules comprises polymers selected from the group consisting of cellulose derivatives such as hydroxypropylmethylcellulose phthalate, hydroxypropyl methylcellulose acetate succinate, hydroxypropyl methylcellulose, carboxymethyl ethyl cellulose, cellulose acetate phthalate and polyvinyl acetate phthalate.

**13.** A composition according to any one of claims 4 to 6, wherein the coating of the third group of microcapsules comprises polymers selected from the group consisting of wax and copolymers of methacrylic acid and methyl methacrylate and/or methyl acrylate that are soluble under pH values higher than 7.

**14.** A composition according to claim 7 characterized in that a first delivery form of the bioactive agent is other than a microcapsule and is gastrosoluble, a second delivery form is microcapsules with an enterosoluble coating and the third delivery form is microcapsules with an enterosoluble coating having dissolution properties in an intestinal or intestinal-like medium such that it releases the bioactive agent in the intestine at slower rate than said second form.

**15.** A composition according to claim 1 for pharmaceutical and /or nutritional use.

**16.** A composition according to claim 15 in the form of a tablet, capsule, gelcap, caplet, chewable tablet, tongue tablet, as a mixture of microparticles in a sachet, or in any adequate nutritive form.

**17.** A composition for oral delivery of vitamin C to a subject, comprising two or three delivery forms of said vitamin C, one, two or three of which being in the form of microcapsules having each an external coating encapsulating vitamin C, characterized in that the coating of the microcapsules of each form is different than that of the other form or forms, said vitamin C being released in the digestive tract in two or three release pulses.

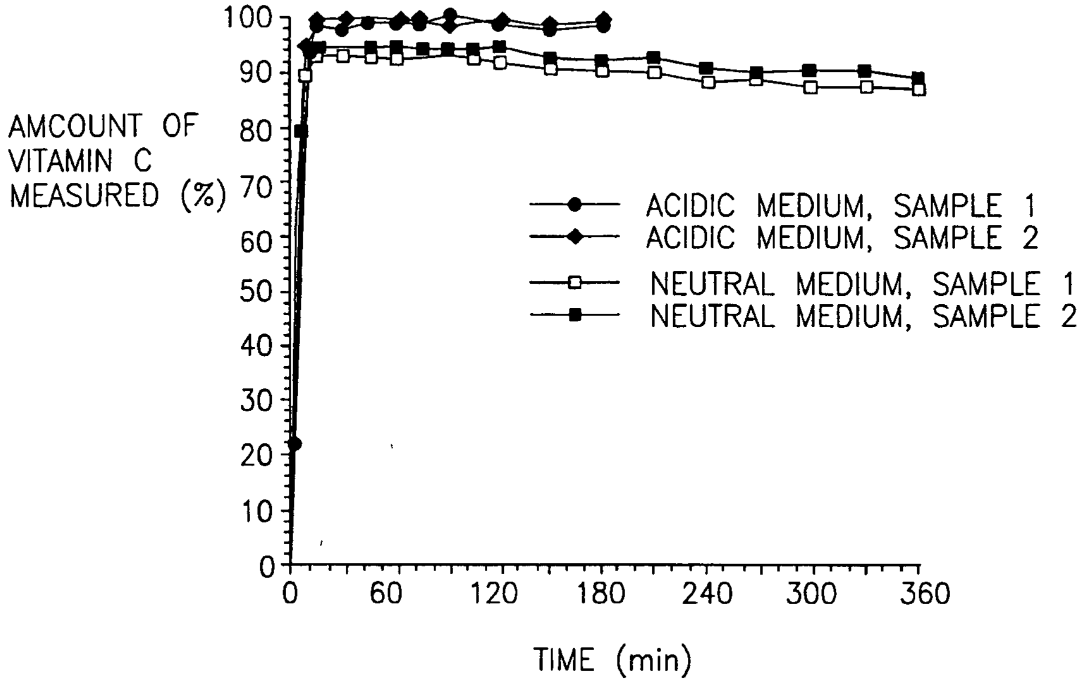

AMCOUNT OF VITAMIN C MEASURED (%)
100
90
80
70
60
50
40
30
20
10
0
0
60
120
180
240
300
360
TIME (min)
ACIDIC MEDIUM, SAMPLE 1
ACIDIC MEDIUM, SAMPLE 2
NEUTRAL MEDIUM, SAMPLE 1
NEUTRAL MEDIUM, SAMPLE 2

FIG.1

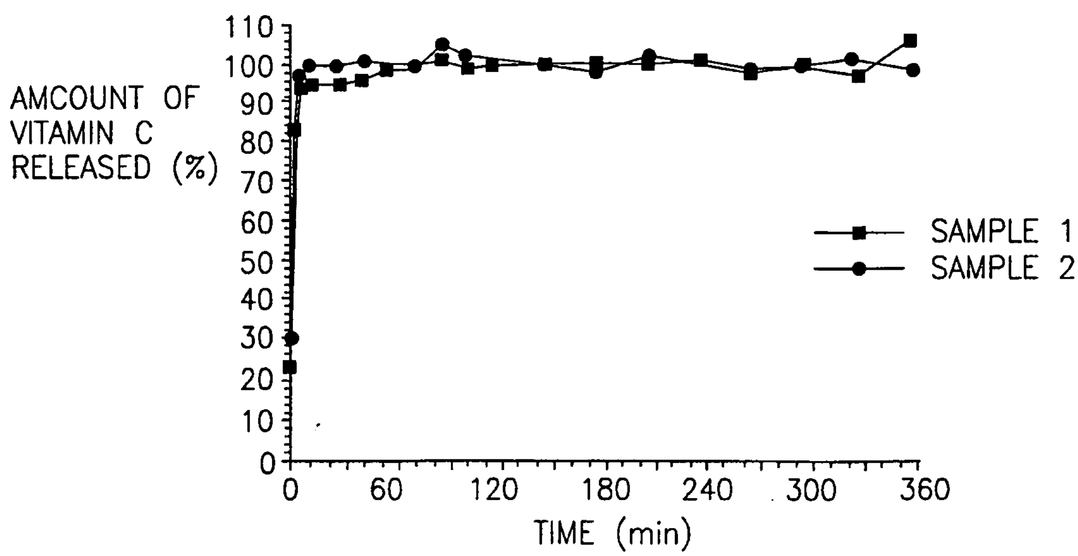
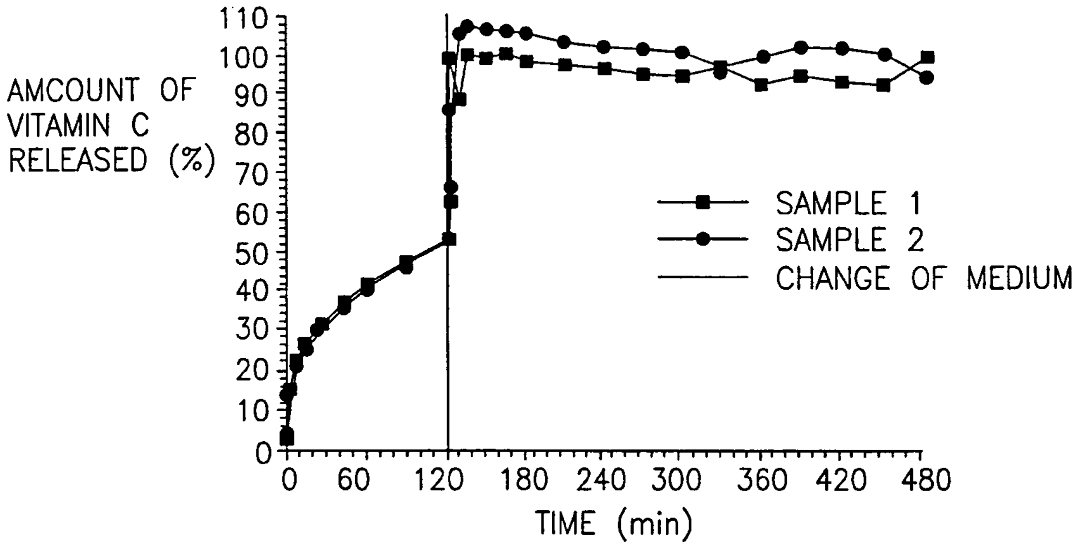
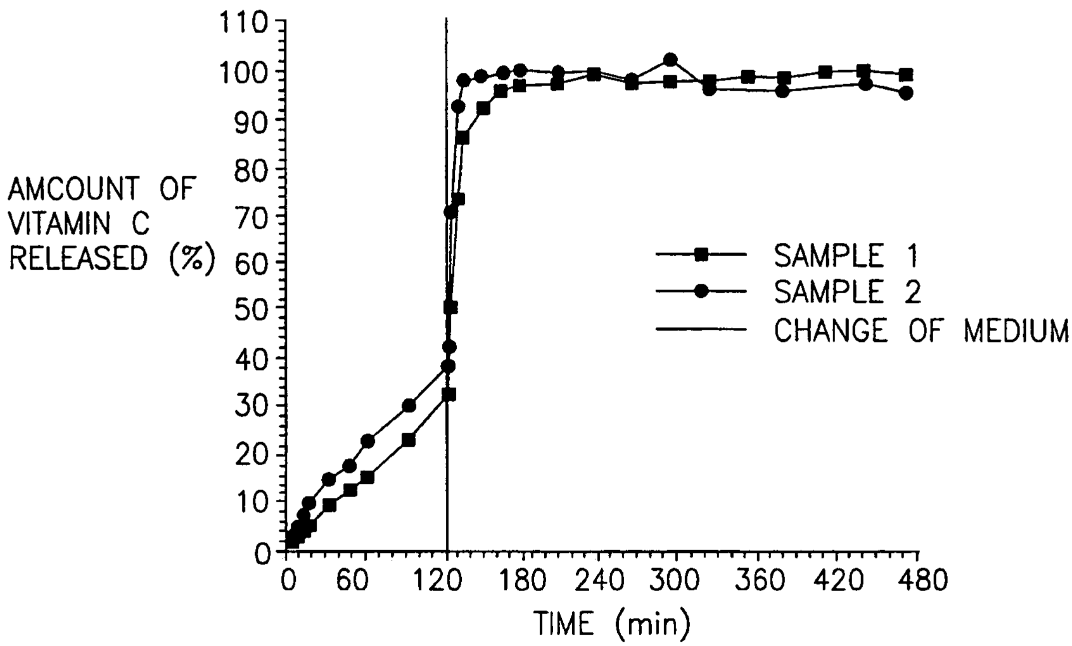

AMCOUNT OF VITAMIN C RELEASED (%)
110
100
90
80
70
60
50
40
30
20
10
0
0
60
120
180
240
300
360
TIME (min)
SAMPLE 1
SAMPLE 2

FIG.2



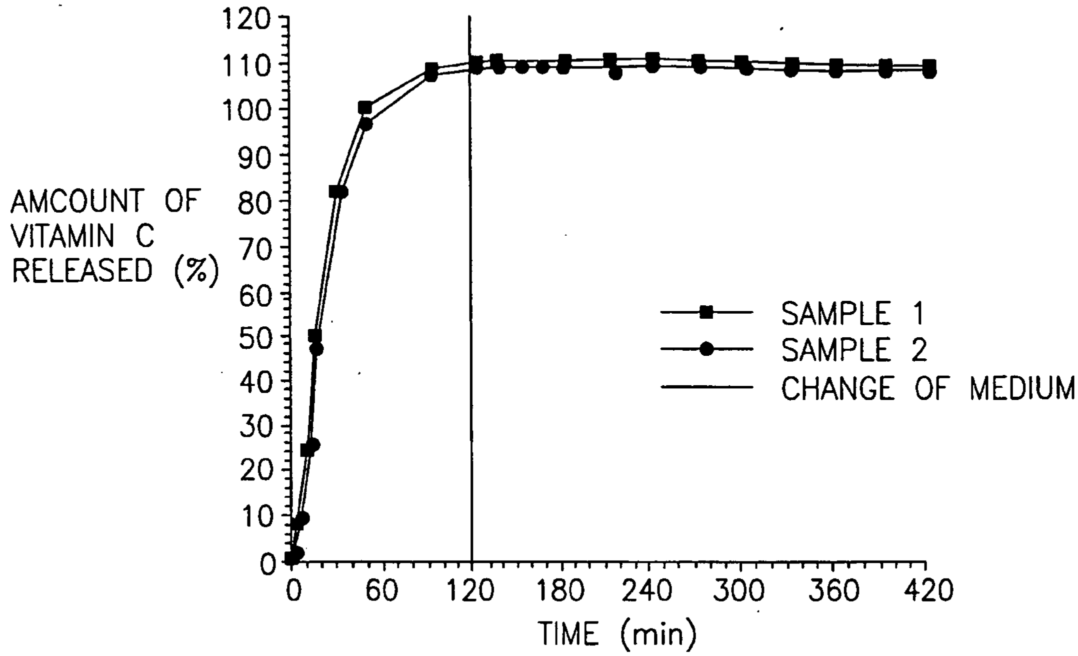
110
100
90
80
70
60
50
40
30
20
10
0
AMCOUNT OF
VITAMIN C
RELEASED (%)
0
60
120
180
240
300
360
420
480
TIME (min)
SAMPLE 1
SAMPLE 2
CHANGE OF MEDIUM


FIG.3



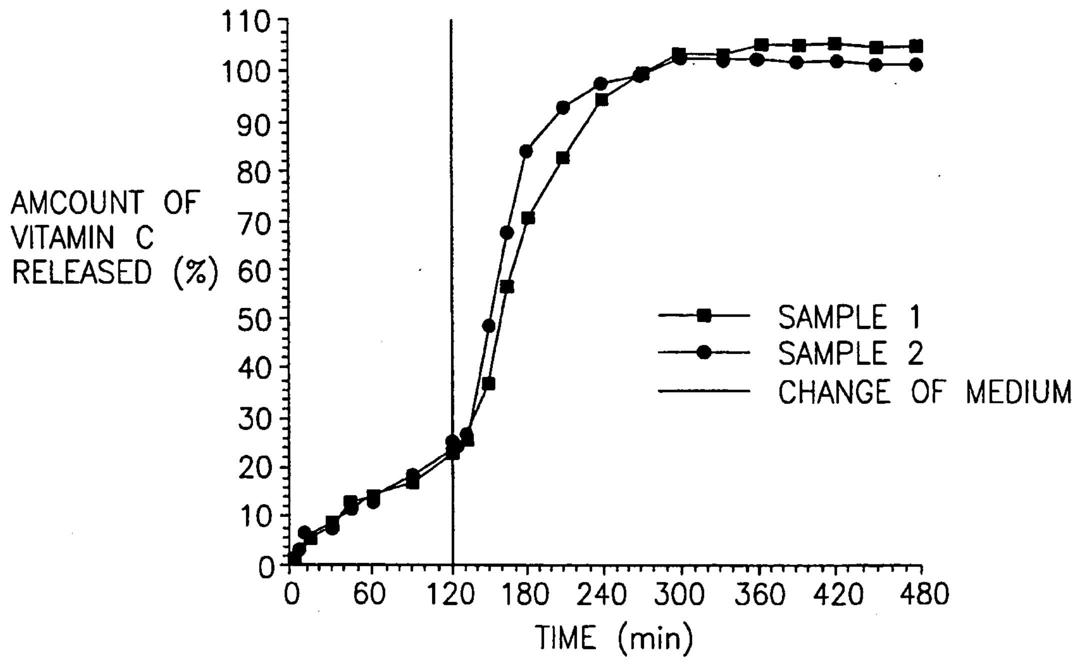
110
100
90
80
70
60
50
40
30
20
10
0
AMCOUNT OF
VITAMIN C
RELEASED (%)
0
60
120
180
240
300
360
420
480
TIME (min)
SAMPLE 1
SAMPLE 2
CHANGE OF MEDIUM


FIG.4



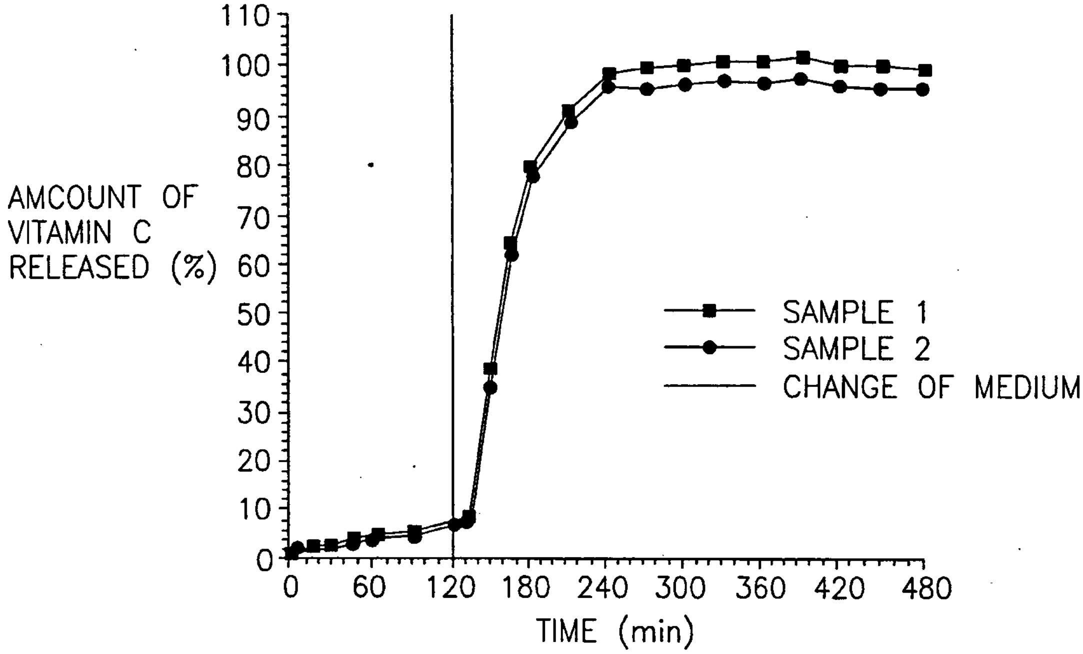
120
110
100
90
80
70
60
50
40
30
20
10
0
AMCOUNT OF VITAMIN C RELEASED (%)
0
60
120
180
240
300
360
420
TIME (min)
SAMPLE 1
SAMPLE 2
CHANGE OF MEDIUM


FIG.5



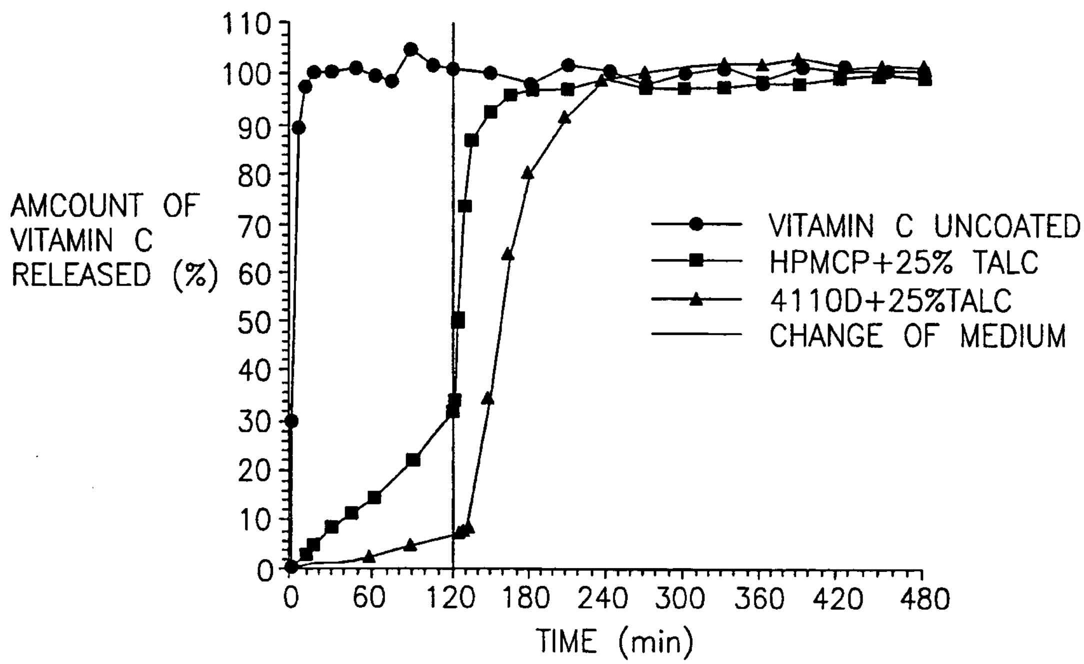
110
100
90
80
70
60
50
40
30
20
10
0
AMCOUNT OF VITAMIN C RELEASED (%)
0
60
120
180
240
300
360
420
480
TIME (min)
SAMPLE 1
SAMPLE 2
CHANGE OF MEDIUM


FIG.6



AMCOUNT OF VITAMIN C RELEASED (%)
110
100
90
80
70
60
50
40
30
20
10
0
0
60
120
180
240
300
360
420
480
TIME (min)
SAMPLE 1
SAMPLE 2
CHANGE OF MEDIUM


FIG.7



AMCOUNT OF VITAMIN C RELEASED (%)
110
100
90
80
70
60
50
40
30
20
10
0
0
60
120
180
240
300
360
420
480
TIME (min)
VITAMIN C UNCOATED
HPMCP+25% TALC
4110D+25%TALC
CHANGE OF MEDIUM


FIG.8

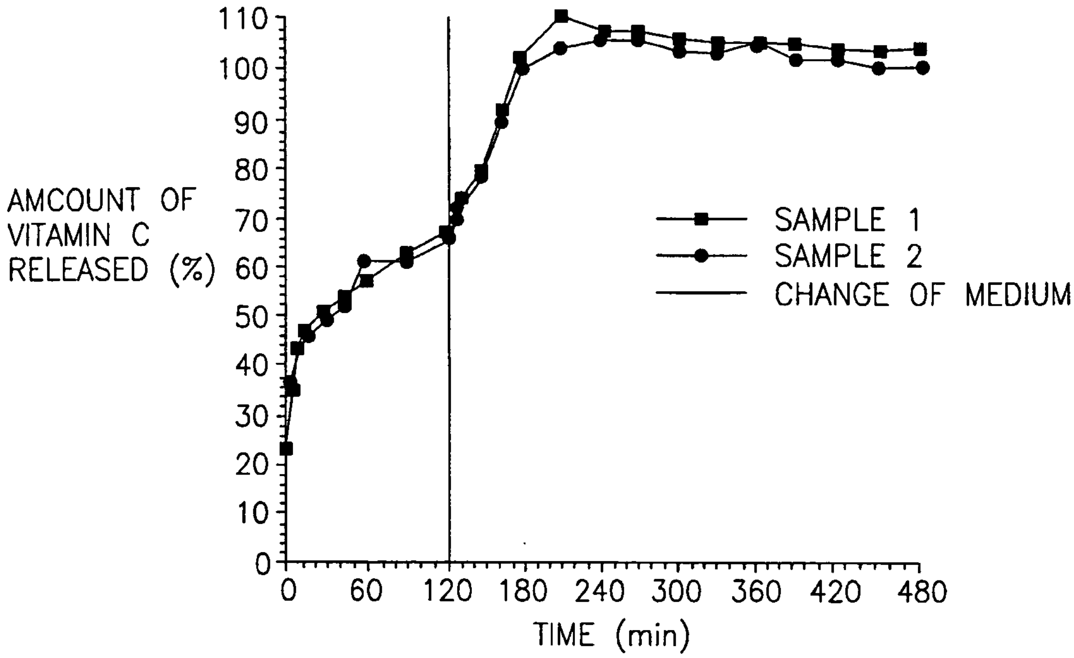

110
100
90
80
70
60
50
40
30
20
10
0
AMCOUNT OF
VITAMIN C
RELEASED (%)
SAMPLE 1
SAMPLE 2
CHANGE OF MEDIUM
0
60
120
180
240
300
360
420
480
TIME (min)

FIG.9

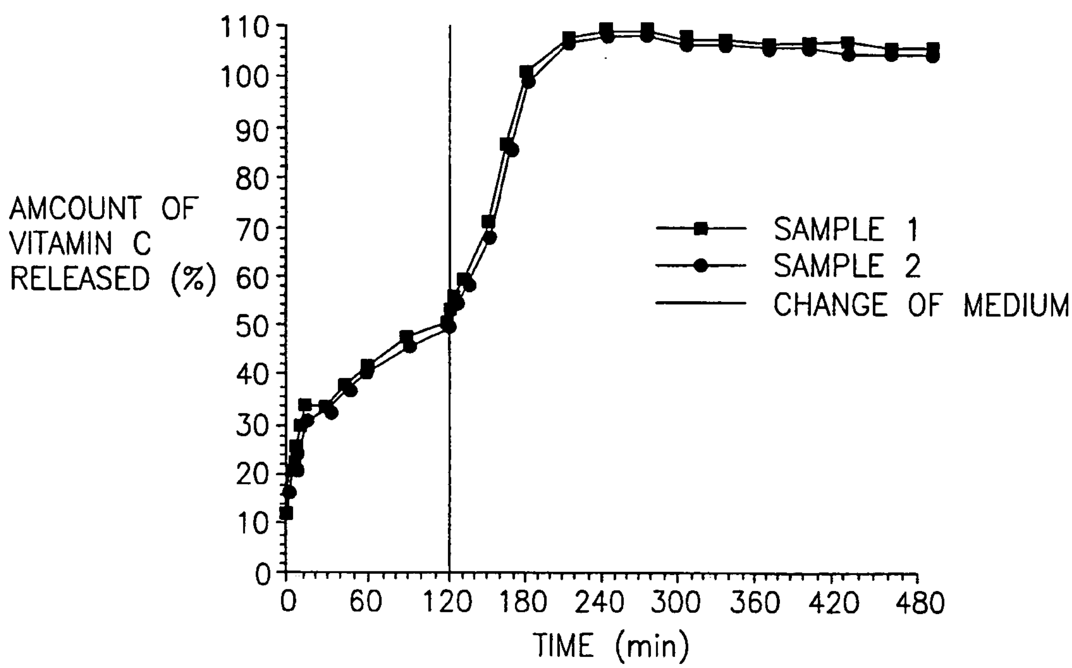

110
100
90
80
70
60
50
40
30
20
10
0
AMCOUNT OF
VITAMIN C
RELEASED (%)
SAMPLE 1
SAMPLE 2
CHANGE OF MEDIUM
0
60
120
180
240
300
360
420
480
TIME (min)

FIG.10

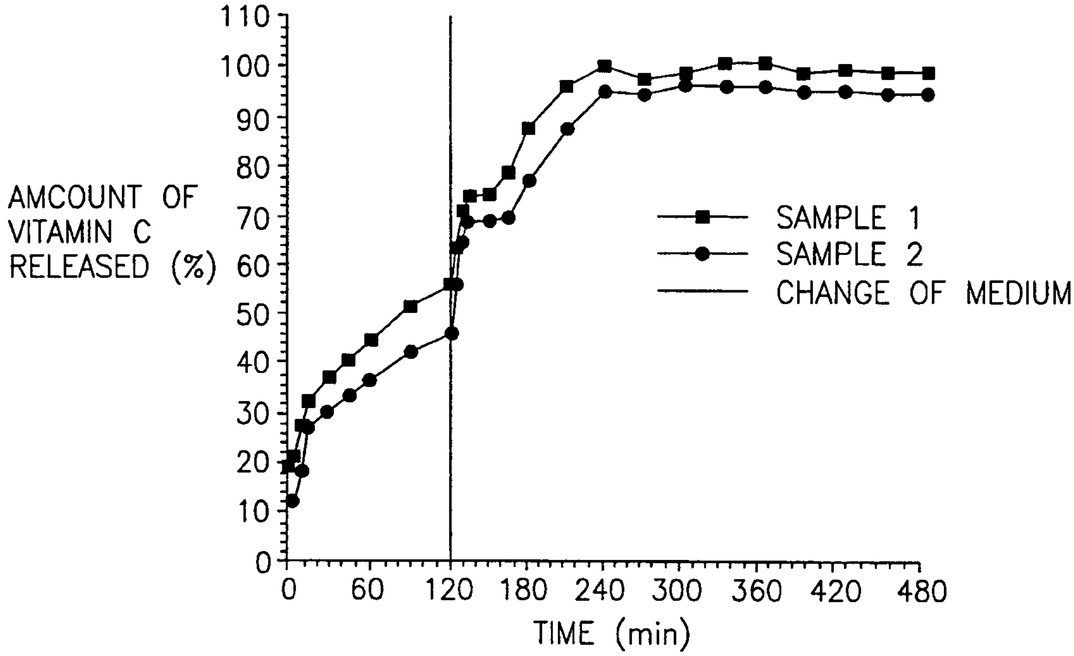

AMCOUNT OF
VITAMIN C
RELEASED (%)
110
100
90
80
70
60
50
40
30
20
10
0
0
60
120
180
240
300
360
420
480
TIME (min)
SAMPLE 1
SAMPLE 2
CHANGE OF MEDIUM

FIG.11

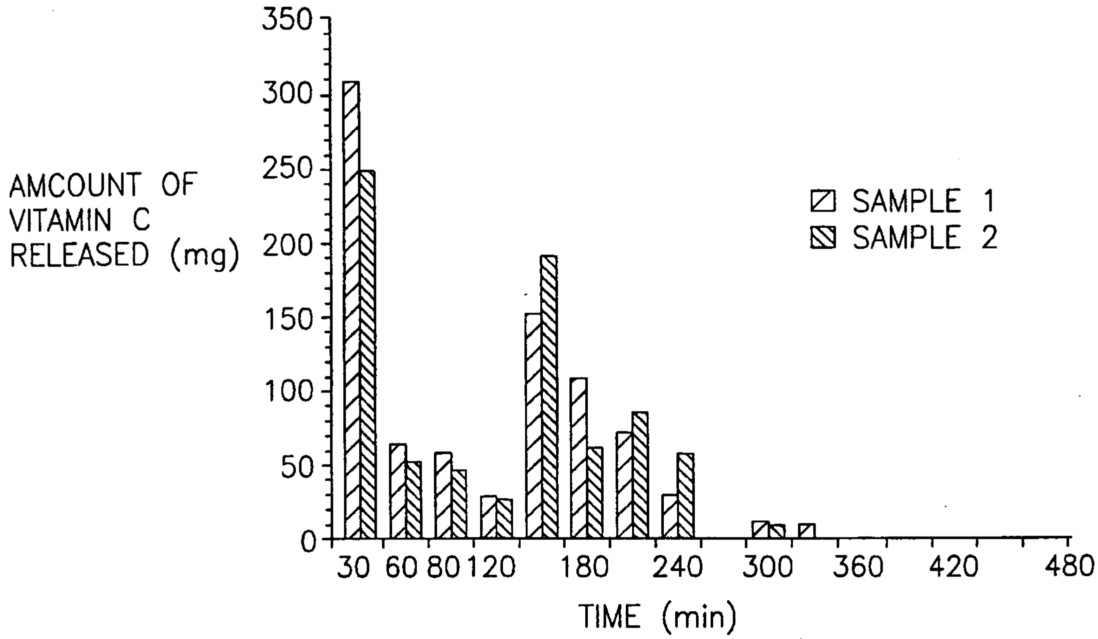

350
300
250
200
150
100
50
0
AMCOUNT OF VITAMIN C RELEASED (mg)
SAMPLE 1
SAMPLE 2
30 60 80 120 180 240 300 360 420 480
TIME (min)

FIG.12

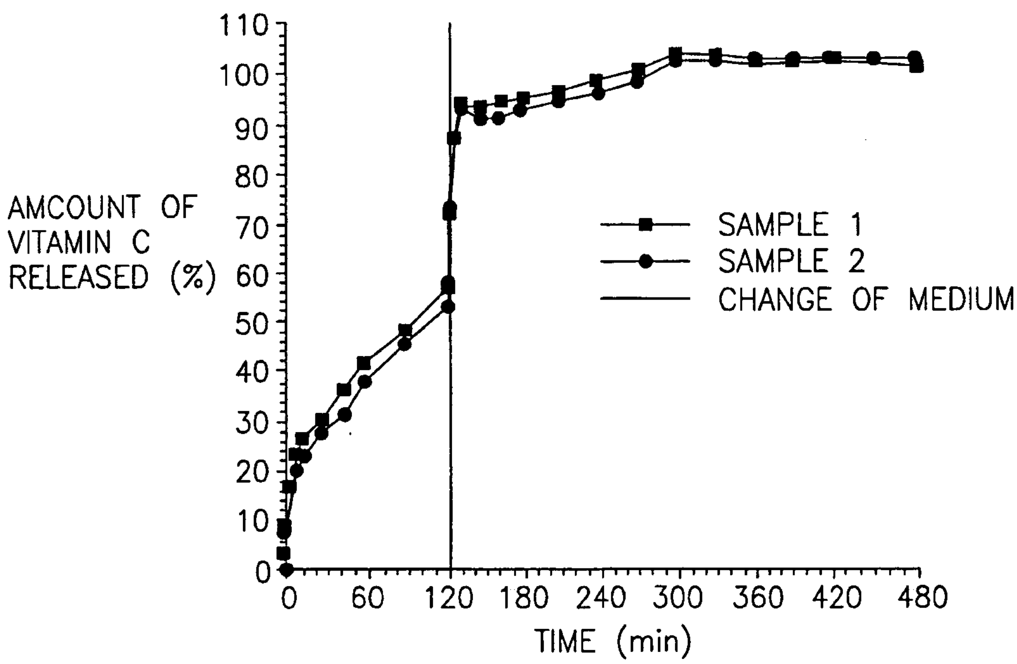

110
100
90
80
70
60
50
40
30
20
10
0
AMCOUNT OF VITAMIN C RELEASED (%)
SAMPLE 1
SAMPLE 2
CHANGE OF MEDIUM
0 60 120 180 240 300 360 420 480
TIME (min)

FIG.13

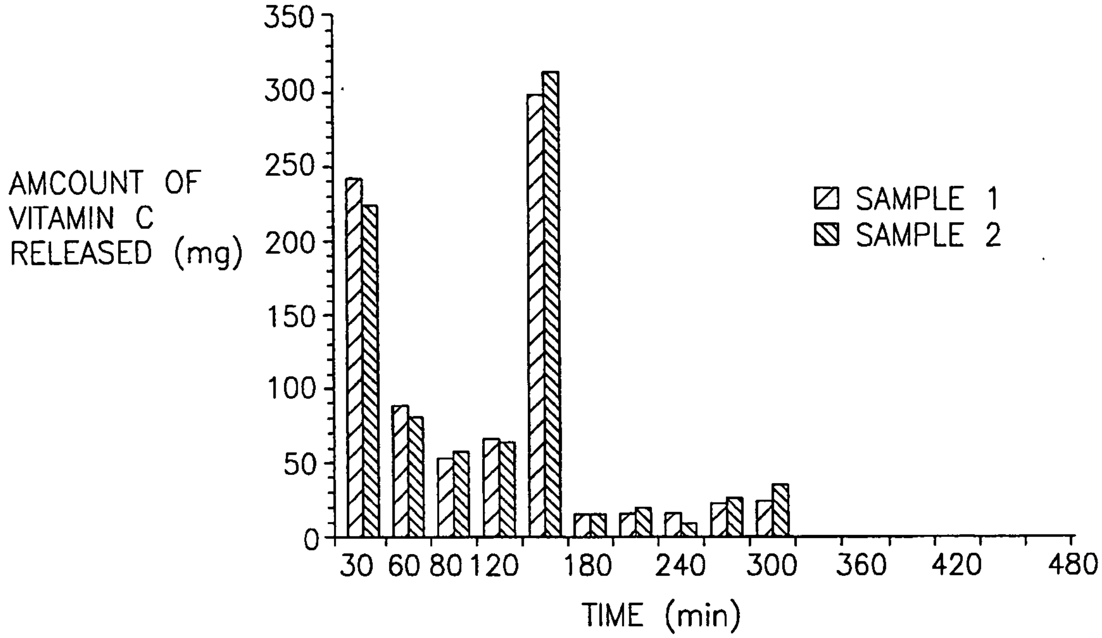

AMCOUNT OF VITAMIN C RELEASED (mg)
350
300
250
200
150
100
50
0
SAMPLE 1
SAMPLE 2
30 60 80 120 180 240 300 360 420 480
TIME (min)

FIG.14

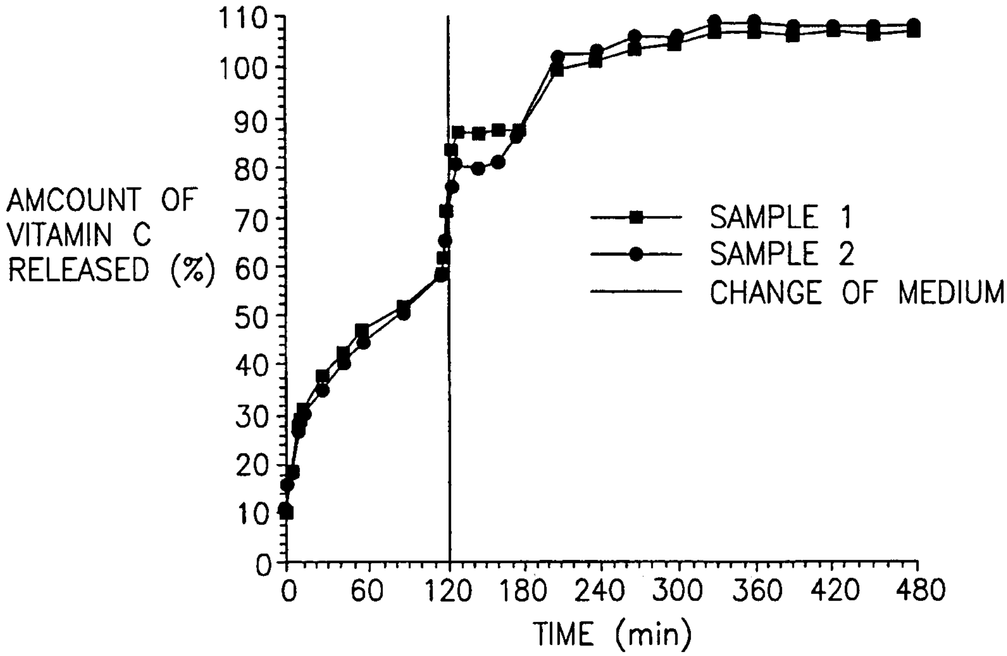
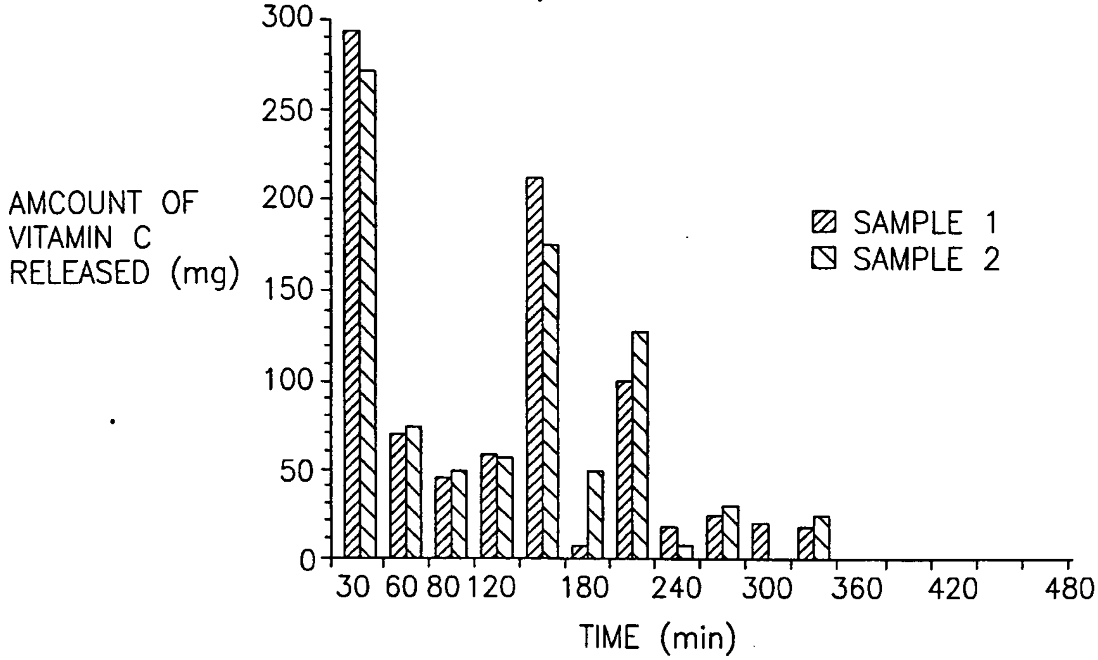
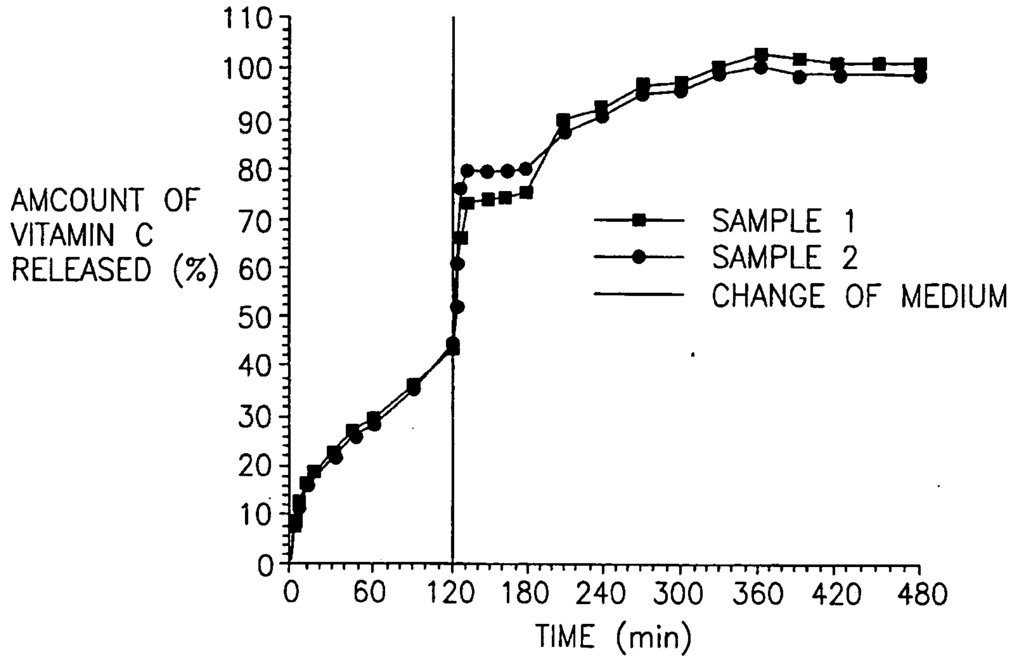

AMCOUNT OF VITAMIN C RELEASED (%)
110
100
90
80
70
60
50
40
30
20
10
0
SAMPLE 1
SAMPLE 2
CHANGE OF MEDIUM
0 60 120 180 240 300 360 420 480
TIME (min)

FIG.15



AMCOUNT OF VITAMIN C RELEASED (mg)
300
250
200
150
100
50
0
30
60
80
120
180
240
300
360
420
480
TIME (min)
SAMPLE 1
SAMPLE 2


FIG.16



AMCOUNT OF VITAMIN C RELEASED (%)
110
100
90
80
70
60
50
40
30
20
10
0
0
60
120
180
240
300
360
420
480
TIME (min)
SAMPLE 1
SAMPLE 2
CHANGE OF MEDIUM


FIG.17

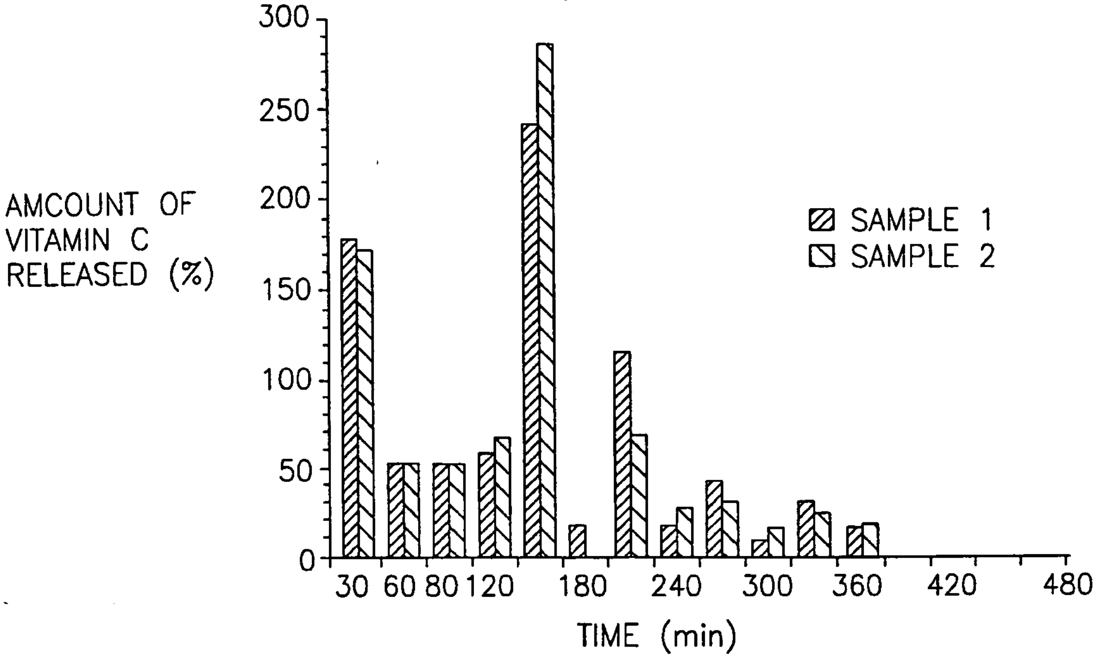

300
250
200
150
100
50
0
AMCOUNT OF VITAMIN C RELEASED (%)
SAMPLE 1
SAMPLE 2
30 60 80 120 180 240 300 360 420 480
TIME (min)

FIG.18

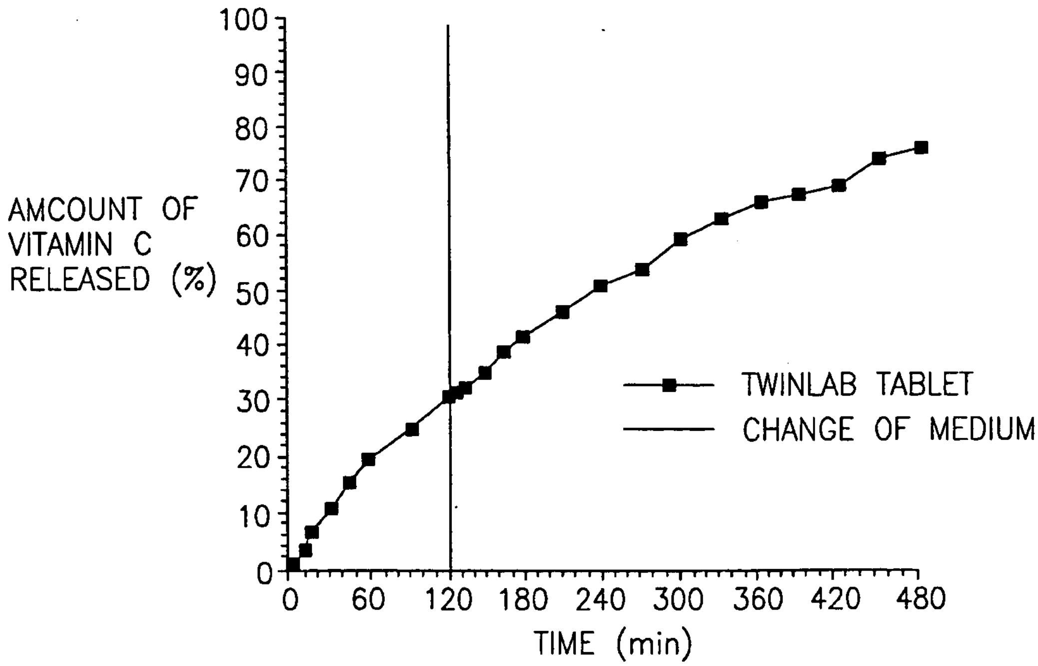

100
90
80
70
60
50
40
30
20
10
0
AMCOUNT OF VITAMIN C RELEASED (%)
TWINLAB TABLET
CHANGE OF MEDIUM
0 60 120 180 240 300 360 420 480
TIME (min)

FIG.19

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 00/00271

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/54 A61K31/375 A23L1/302 A23P1/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | FR 2 157 847 A (ELI LILLY AND COMPANY) 8 June 1973 (1973-06-08)<br>page 13 -page 17; examples 4,5<br>page 14, line 36 -page 15, line 7 | 1,2, 4-11, 14-16 |
| X | WO 98 19667 A (ILEX ONCOLOGY, INC.) 14 May 1998 (1998-05-14) | 1,2, 4-11, 13-16 |
| Y | page 13, line 16 - line 18<br>figure 7<br>page 14, line 9 - line 14<br>figures 14-17<br>page 51 -page 57; example 13 | 3,12 |
| Y | US 5 560 928 A (DEFELICE) 1 October 1996 (1996-10-01)<br>column 6; example 1 | 3 |

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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| Name and mailing address of the ISA<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Authorized officer<br><br>Benz, K |

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